Table 11.1 Comparison of theory and experiment for the mechanosensitive channel, MscL. Experiments measure the pressure difference at which the open probability is 1/2. Comparison between theory and experiment requires knowledge of the pipette radius to convert pressure into tension. (Data taken from E. Perozo et al., *Nat. Struc. Biol.* 9:696, 2002.)

	Theory		Experiment	
n	$\tau_{crit}\,(k_BT/\text{Å}^2)$	$\Delta G(\tau=0)\,(k_BT)$	$P_{1/2}$ (mmHg)	$\Delta G(\tau=0)\,(k_BT)$
16	$2.3\cdot10^{-3}$	5	24 ± 2	4
18	$5.2\cdot10^{-3}$	11.5	42 ± 5	9.4
20	$9.3\cdot10^{-3}$	20.4	72 ± 8	23.5

In addition, the model allows us to probe the free-energy difference at zero tension, which is

$$\Delta G(\tau=0) = G^{\tau=0}_{MscL}(R_0) - G^{\tau=0}_{MscL}(R_c) = \pi\mathcal{K}U^2(R_0 - R_c). \tag{11.63}$$

These results can be compared with the experimental analysis of this problem by appealing to measured values of the various model parameters such as $R_c \approx 23\,\text{Å}$, $R_0 = 35\,\text{Å}$, $U = W/2 - w_n = w_{n=12} - w_n$ and $\mathcal{K} = 0.02\,k_BT/\text{Å}^3$. Using reasonable choices for w_n, the half-width of the undeformed lipid bilayer, for different sized lipids, we obtain $U = 0.65(12 - n)\,\text{Å}$. The theoretical predictions of the model obtained from eqns 11.62 and 11.63 are compared with results of experiments in Table 11.1.

Our discussion of the mechanosensitive channel has laid the groundwork for a more general discussion in Chapter 17 of the important role of ion channels. The present discussion has used simple ideas about membrane elasticity as a window on channel function and our later discussions will shift the emphasis to simple ideas from electrostatics.

11.6 SUMMARY AND CONCLUSIONS

Membranes play a central role in cell biology, separating self from non-self, inside from outside, and distinct specialized compartments within a cell from one another. The study of membranes requires understanding the convergence of a number of different physical principles, ranging from the self-organizing power of the hydrophobic effect to the peculiar properties of two-dimensional fluids to the energetics of membrane bending. Many of the properties of cellular membranes are determined not by their phospholipid constituents but by their many and varied proteins. Some membrane-associated proteins catalyze transport of ions and other molecules across the hydrophobic barrier, while others govern the budding, fusion, and tubule formation processes that are characteristic of the life of membranes inside a cell. The elegant and mutually beneficial interactions between phospholipids and proteins conspire to make cellular membranes lively, but well ordered.

11.7 PROBLEMS

11.1 Membrane protein census

(a) Table 1 of Mitra et al. (2004) reports the mass ratio of proteins and phospholipids in the membranes of various cells and organelles. Use the asserted 2.0 mg of protein for every 1.0 mg of phospholipid in the *E. coli* membrane to compute the areal density of membrane proteins and their mean spacing. Make a corresponding estimate for the membrane of the endoplasmic reticulum using the fact that the mass ratio in this case is 2.6. Explain all of your assumptions in making the estimate.

(b) Dupuy and Engelman (2008) report that the area fraction associated with membrane proteins in the red blood cell membrane is roughly 23%, while the lipids themselves take up roughly 77% of the membrane area. Use these numbers to estimate the number of membrane proteins in the red blood cell membrane and their mean spacing.

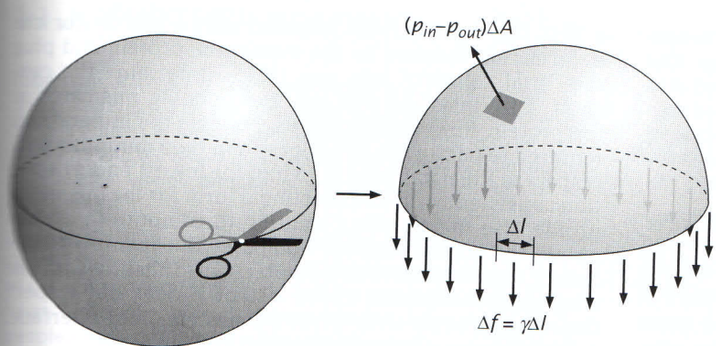

Figure 11.45 Balance of forces in a membrane. The vesicle is cut at the equator and the forces due to the surface tension are replaced with a set of downward acting forces.

Explain all the assumptions you make in constructing the estimate.

11.2 Mathematics of curvature

Consider the function $h(x_1, x_2) = x_1^2 + x_1 x_2 - 2x_2^2$, which we assume describes the shape of a deformed lipid bilayer membrane. As shown in Figure 11.14, x_1 and x_2 are the coordinates of the reference plane below the membrane.
(a) Make a plot of the height as a function of x_1 and x_2.
(b) Compute the principal radii of curvature as a function of x_1 and x_2.
(c) Compute the bending free energy for the piece of membrane corresponding to the square $0 \leq x_1 \leq 1$ and $0 \leq x_2 \leq 1$ in the reference plane.

11.3 Membrane area change

The height function used in the chapter can be used to compute the change in area of a membrane when it suffers some deformation. Consider the height function $h(x, y)$ defined over an $L \times L$ square in the x–y plane. The function $h(x, y)$ tells us the deformed state of the $L \times L$ square. To compute the change in area we divide the square into small $\Delta x \times \Delta y$ squares and consider how each of them is deformed on the surface $h(x, y)$ (see Figure 11.17(A)). The function $h(x, y)$ maps the small square into a tilted parallelogram.
(a) Show that the two sides of the parallelogram are given by the vectors

$$\Delta x \left(\mathbf{e}_x + \frac{\partial h}{\partial x} \mathbf{e}_z \right), \text{ and } \Delta y \left(\mathbf{e}_y + \frac{\partial h}{\partial y} \mathbf{e}_z \right), \qquad (11.64)$$

where $\mathbf{e}_x$, $\mathbf{e}_y$, and $\mathbf{e}_z$ are unit vectors.
(b) The magnitude of the vector product of two vectors is equal to the area subsumed by them, that is, $\mathbf{a} \times \mathbf{b} = ab \sin\theta$, where θ is the angle between vectors $\mathbf{a}$ and $\mathbf{b}$. Using this fact show that the area of the parallelogram is

$$a' = \sqrt{1 + \left(\frac{\partial h}{\partial x} \right)^2 + \left(\frac{\partial h}{\partial y} \right)^2} \, \Delta x \Delta y. \qquad (11.65)$$

(c) Now assume that the partial derivatives appearing in the above equation are small and expand the square-root function using $\sqrt{1 + x^2} \approx 1 + \frac{1}{2} x^2$, to demonstrate the useful formula for the area change,

$$\Delta a = a' - a = \frac{1}{2} \left[\left(\frac{\partial h}{\partial x} \right)^2 + \left(\frac{\partial h}{\partial y} \right)^2 \right] \Delta x \Delta y. \qquad (11.66)$$

By integrating this expression over the entire domain, we can find the total area change associated with the surface. Divide both sides by $a = \Delta x \times \Delta y$ to find a formula for the relative change of area $\Delta a / a$.

11.4 Laplace–Young relation

(a) To derive the Laplace–Young relation (eqn 11.9), imagine a spherical object with internal pressure p_{in} and external pressure p_{out}. This pressure difference leads to an outward pointing normal force at all points on the membrane. Perform the thought experiment shown in Figure 11.45 in which the spherical shell is cut at the equator and the tension in the membrane acts downward to counterbalance the upward force due to the pressure difference. Derive the Laplace–Young relation by balancing the forces acting in the positive z-direction due to the pressure and in the negative z-direction due to the surface tension. Note that the surface tension can be interpreted as a force per unit length acting downward at the equator. Ignore any contribution due to bending energy and focus strictly on the role of the surface tension.
(b) Use energy arguments (rather than force balance arguments) to derive the same result. To do so, consider a small change in the radius of the sphere. As a result of this change in radius, we will incur a free-energy change associated with pV work and a part having to do with γA work. By insisting that the change in free energy for such an excursion be zero obtain the Laplace–Young relation.

11.5 Bending modulus and the pipette aspiration experiment

The pipette aspiration experiment described in the chapter can be used to measure the bending modulus K_b as well as the area stretch modulus. Lipid bilayer membranes are constantly jostled about by thermal fluctuations. Even though a flat membrane is the lowest energy state, fluctuations will cause the membrane to spontaneously bend. The goal of this problem is to use equilibrium statistical mechanics to predict the nature of bending fluctuations and to use this understanding as the basis of experimental measurement of the bending modulus. (NOTE: This problem is challenging and the reader is asked to consult the hints on the book website to learn more of our Fourier transform conventions, how to handle the relevant delta functions, the subtleties associated with the limits of integration, etc.).
(a) Write the total free energy of the membrane as an integral over the area of the membrane. Your result should have a contribution from membrane bending and a contribution from membrane tension. Write your result using the function $h(\mathbf{r})$ to characterize the height of the membrane at position $\mathbf{r}$.

(b) The free energy can be rewritten using a decomposition of the membrane profile into Fourier modes. Our Fourier transform convention is

$$h(\mathbf{r}) = \frac{A}{(2\pi)^2}\int \tilde{h}(\mathbf{q})e^{-i\mathbf{q}\cdot\mathbf{r}}\,d^2\mathbf{q}, \tag{11.67}$$

where $A = L^2$ is the area of the patch of membrane of interest. Plug this version of $h(\mathbf{r})$ into the total energy you derived above (that is, bending energy and the energy related to tension) to convert this energy in real space to an energy in q-space. Note that the height field in q-space looks like a sum of harmonic oscillators.
(c) Use the equipartition theorem in the form $\langle E(\mathbf{q})\rangle = k_BT/2$, where $E(\mathbf{q})$ is the energy of the qth mode and the free energy can now be written as

$$F[\tilde{h}(\mathbf{q})] = \frac{A}{(2\pi)^2}\int E(\mathbf{q})d^2\mathbf{q}. \tag{11.68}$$

Use this result to solve for $\langle|\tilde{h}(\mathbf{q})|^2\rangle$ which will be used in the remainder of the problem.
(d) We now have all the pieces in place to compute the relation between tension and area and thereby the bending modulus. The difference between the actual area and the projected area is

$$A_{act} - A = \frac{1}{2}\int(\nabla h(\mathbf{r}))^2\,d^2\mathbf{r}. \tag{11.69}$$

This result can be rewritten in Fourier space as

$$\langle A_{act} - A\rangle = \frac{1}{2}\frac{A^2}{(2\pi)^2}\int_{\pi/\sqrt{A}}^{\pi/\sqrt{a_o}} q^2\langle|\tilde{h}(\mathbf{q})|^2\rangle 2\pi q\,dq. \tag{11.70}$$

Work out the resulting integral which relates the areal strain to the bending modulus, membrane size, temperature, and *tension*. The limits of integration are set by the overall size of the membrane (characterized by the area A) and the spacing between lipids (a_0 is the area per lipid), respectively. This result can be directly applied to micropipette experiments to measure the bending modulus. In particular, using characteristic values for the parameters appearing in the problem suggested by Boal (2002) such as $\tau \approx 10^{-4}$ J/m^2, $K_b \approx 10^{-19}$ J and $a_0 \approx 10^{-20}$ m^2 show that

$$\frac{\Delta A}{A} \approx \frac{k_BT}{8\pi K_b}\ln\left(\frac{A\tau}{K_b\pi^2}\right), \tag{11.71}$$

and describe how this result can be used to measure the bending modulus. (For an explicit comparison with data, see Rawicz et al., 2000. For further details on the analysis, see Helfrich and Servuss, 1984. An excellent account of the entire story covered by this problem can be found in Chapter 6 of Boal, 2002.)

11.6 Variational approach to deformation induced by MscL

In the chapter, we explicitly minimized eqn 11.35 by solving a partial differential equation for the unknown deformation field $u(x)$. As an alternative, consider the powerful technique of adopting a variational solution. As a first try, consider the function $u(x) = Ae^{-x/x_0}$ for the deformation induced by the membrane protein and plug this "trial solution" into eqn 11.35. Determine the constant A by insisting that the hydrophobic mismatch at the protein–lipid interface is u_0. Perform the integrations required to obtain the free energy and then minimize with respect to the parameter x_0 to find the lowest energy solution consistent with this functional form for the mismatch profile. Use the resulting solution to compute G_h and to obtain the critical tension. Compare your result with the "exact" result found in the chapter. One difficulty with the trial solution adopted above is that it does not respect the condition that $u'(x) = 0$ at the protein–lipid interface. A more appropriate trial solution is of the form

$$u(x) = u_0\left(1 + \frac{x}{x_0}\right)e^{-x/x_0}. \tag{11.72}$$

Verify that this trial solution satisfies the boundary conditions at the lipid–protein interface. Plug this trial solution into the free energy and minimize with respect to x_0 and find the free-energy-minimizing deformation profile. Use this result to plot the total free energy as a function of radius for several different tensions including the critical tension (that is, produce a figure analogous to Figure 11.44). How does the critical tension compare with the value obtained using the full solution of the partial differential equation?

11.7 Two-dimensional Treatment of MscL

Consider a two-dimensional generalization of eqn 11.35.
(a) Show that the free energy associated with membrane deformation may be written as

$$G_{hydrophobic} = \frac{K_b}{2}\int(\nabla^2 u)^2 d^2r + \frac{K_t}{2}\int\left(\frac{u}{a}\right)^2 d^2r$$

To this add the change in free energy due to the change in tension, namely,

$$G_{tension} = G_0 - \tau\pi R^2, \tag{11.73}$$

where G_0 is a constant that sets the reference value for the energy of the loading device. Explain why the free-energy expression for the composite system of channel and loading device can now be written as

$$G_{MscL} = G_0 - \tau\pi R^2 + \frac{K_b}{2}\int(\nabla^2 u)^2 d^2r + \frac{K_t}{2}\int\left(\frac{u}{a}\right)^2 d^2r. \tag{11.74}$$

To compute this free energy explicitly, we need to know the profile of the field $u(\mathbf{r})$ around the membrane.
(b) Find the Euler–Lagrange equation for this free energy functional.
(c) Solve the partial differential equation for the deformation profile due to the membrane protein and imitate the various steps leading up to eqn 11.57, but now done for the full two-dimensional deformation profile.
(d) Compare this solution with that of the one-dimensional approximation given in the chapter.

11.8 FURTHER READING

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